

## Landmark - Open2Dprot sample landmark creation/editing program

Welcome To Landmark

<http://www.ccrnp.ncifcrf.gov/Open2Dprot/Landmark/>  
and  
<http://open2dprot.sourceforge.net/Landmark>

Note: This pre-alpha level software is under construction and will change. Not all functionality described is fully implemented or fully debugged. This documentation will undergo revision as the program develops.

## Introduction

Landmark is a Open2Dprot project Java 2D spot program to Landmark samples into the system for the Composite Sample Database (CSD). The CSD consists of protein expression per (spot) for N samples may be constructed from N sample spot lists. Landmark is a program to aid in the construction of a database describing the sample information, region of interest and grayscale calibration (if applicable for the samples being used).

Landmark is a step [1] module in pipeline analysis for the Open2Dprot project.

You can currently download the pre-alpha version and install it on your computer. Currently, Landmark is hardwired to start with the demo samples and with the -gui switch. The remainder of this home page contains links to some screen shots of the interactive GUI. The Web site contains some initial (rudimentary) documentation.

See the Reference Manual for details. You read about downloading and installing the program on your computer. The source code will be put onto open2dprot.sourceforge.net when it is a bit more stable - currently it undergoing major refactoring.

## Examples - samples of screen shots

To give the flavor of running the spot pairing, we provide a few screen shots of the graphical user interfaces and some results. You can these images in the list below or view all of the screen shots in a single Web page.

- Initial user interface Report Window
- Initial command line options tool

Please contact us with suggestions and comments. If you make interesting changes in the source code, please send us a copy and describe your changes so we can merge them in the released version.

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*Contact  
us*

Landmark is a contributed program available at  
Open2Dprot.sourceforge.net/Landmark  
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Revised:  
10/04/2005

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Landmark Program Contributors

## Landmark Program Contributors

The current Landmark program is the result of a collaborative effort with contributions from various groups. The current Java program is partly based based on some of the code refactored from the C/Unix GELLAB-II 2D gel landmarking program (landmark, 1993 version). That in turn had been refactored from a DEC-10/SAIL language version. The new Java version also includes code from the open source Seg2Dgel, CmpSpots, Accession, and Flicker, and MAExplorer programs.

Many individuals have been involved over the years in its development both as direct collaborators running 2D gel experiments as well as in offering suggestions for improvement including: Lew Lipkin, Eric Lester, Jim Myrick, Mary Robinson, Carl Merrill, Heinz Busse, Peter Sonderegger, Pete Rogan, Ye Chung Wu, Steve Aley, Mike Alley, Rob Ashmore, Kyle Upton, Jeff Orr, Mort Schultz, George Carman, Mark Miller, Arthur Olson, Norman and Leigh Anderson, Sam Hanash, Jian Li, and many others in the electrophoresis community. Published descriptions of the algorithms for the GELLAB-II program on the Open2Dprot Web site and are in the References section of this Web site.

The core group developing the Landmark program source currently consists of

- \* Peter Lemkin
- \* Greg Thornwall
- \* Jai Evans

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us*

Landmark is a contributed program available at  
[open2dprot.sourceforge.net/Landmark](http://open2dprot.sourceforge.net/Landmark)

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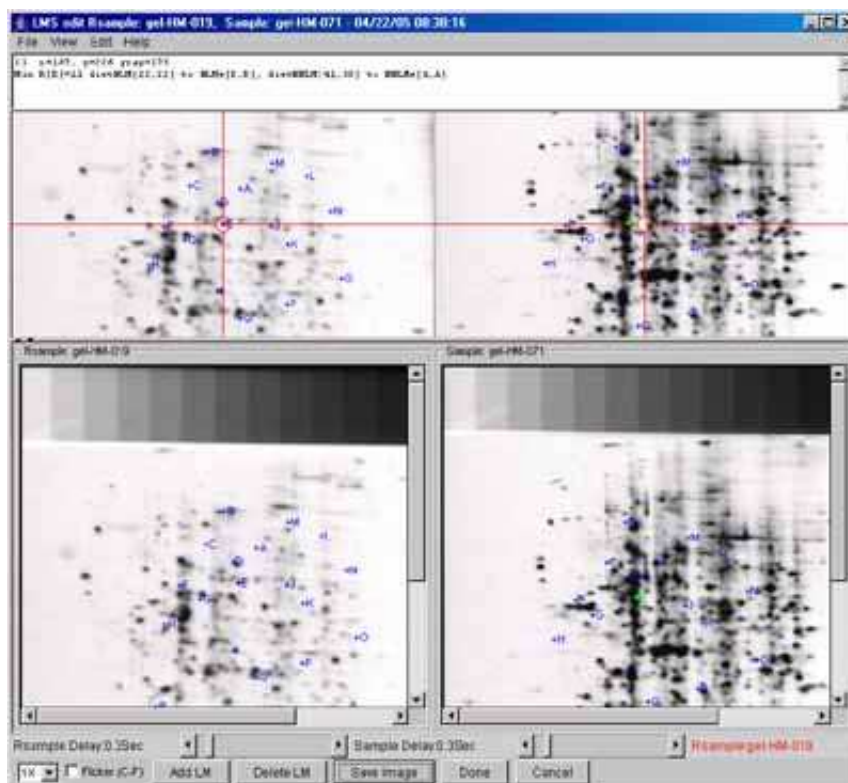
Hosted at Open2Dprot  
open2dprot.sourceforge.net

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Contact us

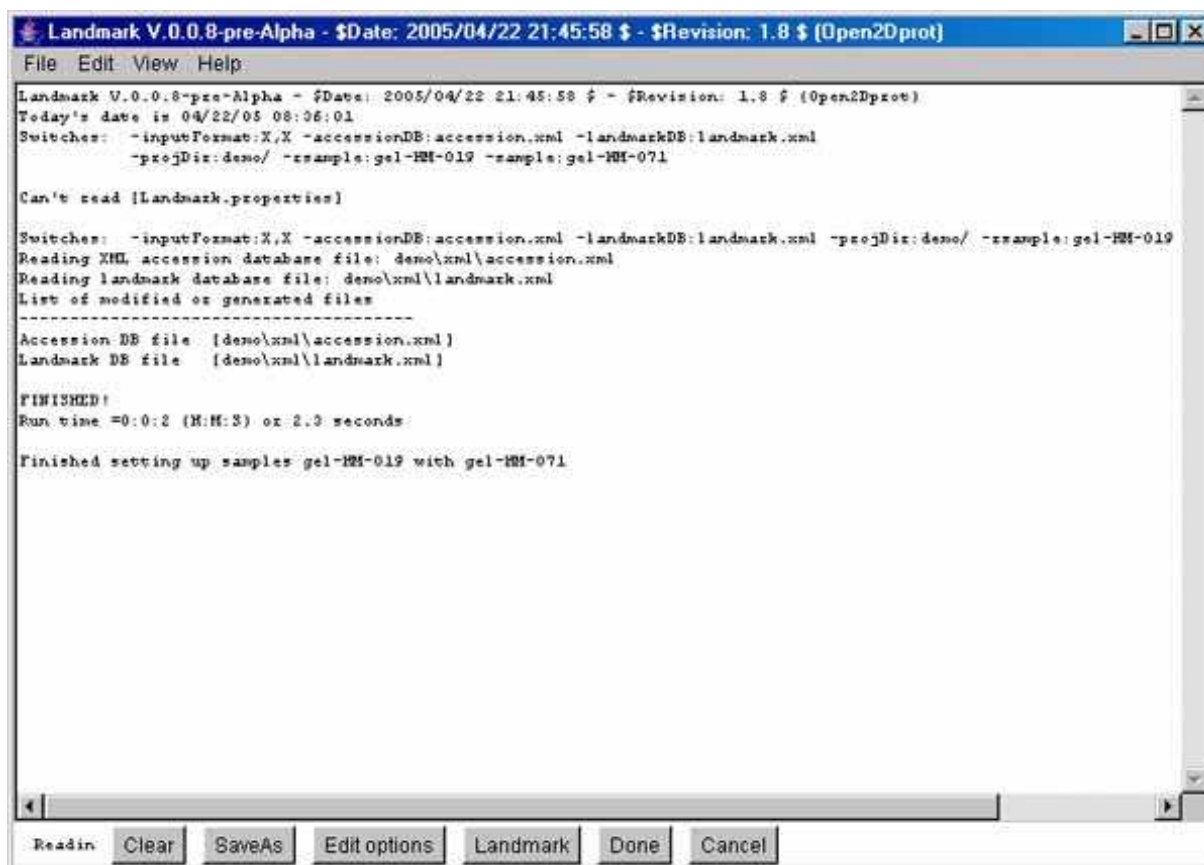
Revised: 05/19/2006

Landmark Reference Manual



## Landmark Reference Manual

The following description will tell you what how to use the 2D image sample Landmark program to create and edit sets of landmark coordinates between a reference sample (Rsample) and another sample (Sample). For N samples, normally one is defined as the Rsample and the remaining N-1 samples as Samples. These landmark sets are generated in N-1 sessions of using this program. It also discusses the startup options and how to run the program. Figure 1 shows a screen view of the initial Landmark reporting window.



**Figure 1. Screen view of the Landmark program Report Window interface** This screen shot shows the initial Landmark Report Window graphical user interface available after the program starts. There are a number of pull-down menus (File, Edit, View, and Help) as well as pushbuttons at the bottom of the window. **Edit Options** lets you change the startup options. The **Landmark** button causes the interactive Landmark Editor window to pop up. When you are finished editing a landmark set, pressing the **Done** button will save the data set in the landmark database file. You can also do these operations from the menus.

## 1. Introduction

Landmark is an open source Java 2D sample Landmarking program for creating landmark sets for samples paired with a reference sample (Rsample) into the Open2Dprot database. It is part of the Open2Dprot project (<http://open2dprot.sourceforge.net/>). Landmark is similar to and derived from an earlier landmark program in the GELLAB-II 2D gel spot pairing program **landmark** described in the GELLAB-II reference manual. Landmark has been generalized to other types of samples besides 2D gels. While the original program was written in C, Landmark is written in Java, uses XML input and output files and has an Java-based graphical user interface. This initial open-source Landmark program code could be used as the basis for more advance spot pairing methods. Currently the Landmark file is `landmark.xml` and is stored in the project "project"/xml/ directory.

The program is run interactively (-gui). After the spot pairing is finished, the user has the option of interactively examining the paired spot data overlayed on the original sample images. The user may also modify the input switch options and save the new options in a "Landmark.properties" file in the current project directory when they exit so that the last used options may be used as the default switch options in subsequent running of Landmark.

## Project directory structure for Open2Dprot and Landmark

All Open2Dprot programs assume a project directory structure. This must exist for the program to proceed. You can either create the structure prior to running any of the programs or you can create it on the fly using the `-projDir:user-project-directory`. It will lookup and/or create the following sub-directories inside of the *user-project-directory*.

```
batch/ directory holding temporary batch files - [NOT USED by Landmark]
cache/ directory holding temporary CSD cache files - [NOT USED by Landmark]
ppx/  directory holding your original gel input files
rdbms/ directory holding CSD database RDBMS files - [NOT USED by Landmark]
tmp/  directory holding generated sample image files
xml/  directory that holds  Accession DB, landmark DB,
      SSF sample spot-list files, and SPF samples paired-spot-list files
```

The use of these directories is discussed in the rest of this document.

## Landmark database file

The sample and reference samples to be paired is specified by its sample name using the `-sample` and `-rsample` command line switches. It can also be specified at run time using the (**File | Open Rsample | ...**) and (**File | Open Sample | ...**) menu commands.

The application looks up the sample in the accession database (in `xml/accession.xml` or as specified using the `-accessionFile` switch) and gets additional information about the sample. The Open2Dprot <http://open2dprot.sourceforge.net/Accession> pipeline module is used for entering samples into the accession database.

The images associated with the samples can be used with the landmark editor. These images may be in TIFF (.tif, .tiff), JPEG (.jpg), GIF (.gif), or PPX (.ppx GELLAB-II) format. TIFF images may be 8-bits/pixel through 16-bits/pixel, whereas JPEG, GIF, and PPX are 8-bit images. Gray values in the image files have black as 0. This is mapped to 0 for white and the maximum pixel value for black.

The input sample image files, if any, are kept in the *user-project-directory/ppx/* sub-directory. This database directory structure is consistent with and is used by the other Open2Dprot analysis pipeline programs.

## 2. Running Landmark and specifying parameter options via the command line

The program is run interactively (`-gui`) with a graphical user interface (GUI). The user may also modify the input switch options and save the new options in a "Landmark.properties" file in the current project directory so that it may be used as the default switch options in subsequent running of the program. [Status: the Landmark.properties file is not enabled.] All options including the input reference sample and other sample to be paired are specified via GNU/Unix style switches on the command line (`-switch{optional ':parameters'}` and its negation as `-noswitch`). However, if GUI mode is used, you can interactively specify the switches and their options. It is assigned previously by software that generated the SSF spot lists.

## Local Folders and files created and used by Landmark

When Landmark is first started, it will check for the following folders and files in the installation directory and create them if they can not be found.

- **Landmark.properties** - is the default command line switch options to be used (if present). You can edit the default switch options and then save them into the Landmark.properties file.

Normally the project directory is setup prior to running Landmark and it expects the data to be in the following directories. You can also use the `-projDir:user-project-directory` switch to specify a (possibly new) project directory.

- **batch/** directory holding temporary batch files - [NOT USED by Landmark]
- **cache/** directory holding temporary CSD cache files - [NOT USED by Landmark]
- **ppx/** directory holding your original gel input files
- **rdbms/** directory holding CSD database RDBMS files - [NOT USED by Landmark]
- **tmp/** directory holding generated sample image files
- **xml/** directory that holds Landmark DB, landmark DB, SSF spot-list files, and generated SPF paired spot list file generated by Landmark

## Landmark command-line arguments switch usage

The command line arguments usage is:

```
Landmark -rsample:Reference-sample -sample:sample [< optional switches >]
```

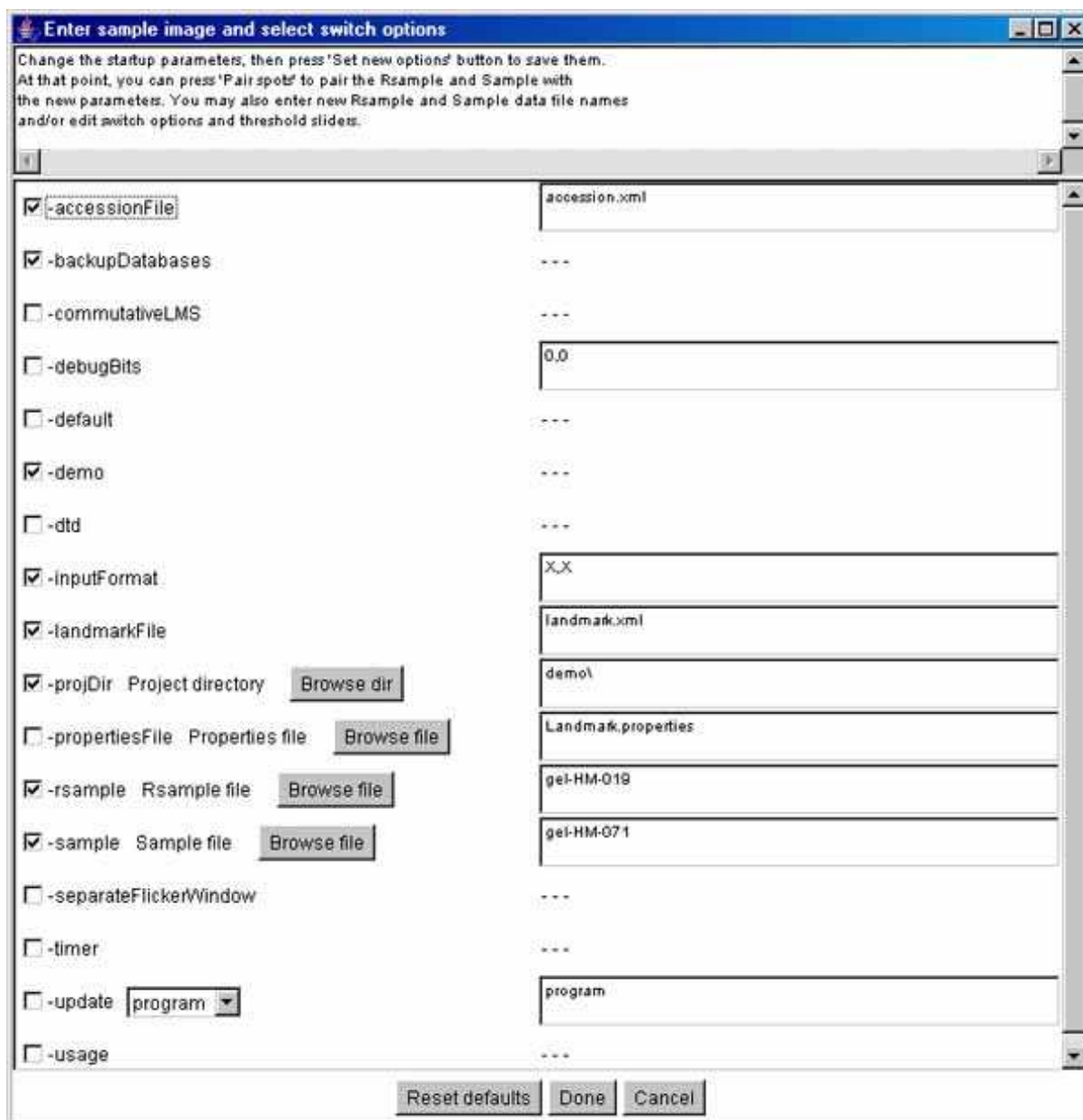
The complete list of switches is given later in this manual and as well as some examples of typical sets of switches. The user defined default switches may be specified as a resource string 'Landmark.properties' file saved in the project directory. For example:

```
Landmark -rsample:gel-HM-19 -sample:gel-HM-071 -project:demo/ -gui
```

## Options wizard window for setting the command line switches

If you invoke the **Edit options** button in the Report window (or from the Edit menu), it will popup an options wizard shown in Figure 2 to let you set or change the switch options and then to save these as the new default switch options. The default is saved in the `Landmark.properties` file when you exit program.





**Figure 2. Screen view of the popup options wizard window for setting the command line switches, parameter and specifying input samples to be paired.** All of the switches are available in the scrollable window. Switches are checked if they are enabled and if the switch requires a value, the current value is shown in the data entry window to its right. On the right there may be several threshold sliders for the upper sizing values for several parameters. In the middle, are several Browse buttons to use for specifying a different samples (**-rsample:** and **-sample:**), and directories. Clicking on any switch will show a short help message associated with that switch at the top of the window. Pressing the **Set new default** button will pass the new options values back to Landmark. Note: for this to take effect, you must exit and then restart Landmark. Then to use them, press the **Pair spots** button in the main Report Window.

## Updating Landmark from the Open2Dprot Web server using -update switch

As new versions of Landmark are developed and put on the Web server, a more efficient way of updating your version is to use the -update commands. There are four options:

-update: <b>program</b>	to update the program jar file
-update: <b>demo</b>	to update the demonstration files
-update: <b>doc</b>	to update the documentation files
-update: <b>all</b>	to update all of the above

After updating the program, it should be exited and restarted for the new program to take effect.

## Increasing the allowable memory used by Landmark

If you are working with very large images that require a lot of memory, you might want to increase the memory available at startup.

If you are using the Windows Landmark.exe file or clicking on the Landmark.jar file, you can't change the default startup memory.

If you are using the Landmark.jar in a script using the java interpreter as in the following example which uses the **-Xmx256M** (specifying using 256Mbytes at startup). Change 256 to a larger size if you want to increase startup memory.

```
java -Xmx256M -jar Landmark.jar {additional command line args}
```

## 3. Command and Report Window - the command center

Landmark is designed to be used with the graphical user interface (GUI) which creates a Report Window that captures a report of the spot pairing output as well as additional output directed to it by the user. There are a set of pull-down menus as well as a set of buttons for often used functions.

All logged output is sent to the report window in a scrollable text window that may be saved or used for cut and paste operations. A set of command buttons at the bottom of the window are replicates of commands in the menus, but are easier to access. They include the following functions:

- **Status area** - reports the current status of the processing.
- **Clear** - clear the report screen
- **SaveAs** - save this report screen in a text file (user is prompted for the file name)
- **Edit options** - popup an Options Window to let the user set/clear switches, assign switch parameters (if any), adjust the threshold sizing limits, and specify input samples.
- **Landmark** - popup the Landmark information popup editor
- **Done** - Save Landmark database changes if required. Then save the `Landmark.properties` file, and exit the Landmark program.
- **Cancel** - exit the program. If you need to save changes, ask the user first. If they say no, do not save the changes. Save the `Landmark.properties` file, and exit the Landmark program.

## 4. Pull-down menus in the Landmark Report Window

The menu bar at the top of the Report Window (see Figure 1) contains four menus. There are also some command buttons at the bottom of the window for convenience that are also menu commands.

1. File menu - to open the samples to be paired and other file operations.
2. Edit menu - to change invoke various editing and other options including command line switches, computing window, and memory size
3. View menu - to view Landmark data from the Landmark database.
4. Help menu - popup Web browser documentation on the Landmark program.

## Menu notation

In the following menus, selections that are *sub-menus* are indicated by a 'Indicates that the menu has a submenu '. Selections prefaced with a 'Checkbox on ' and indicate 'Checkbox off ' indicate that the command is a checkbox that is enabled and disabled respectively. Selections prefaced with a 'Radio button on ' and indicate 'Radio button off ' indicate that the command is a multiple choice "radio button" that is enabled and disabled respectively, and that only one member of the group is allowed to be on at a time. The default values set for an initial database are shown in the menus. Selections that are not currently available will be grayed out in the menus of the running program. The command short-cut notation **C-key** means to hold the Control key and then press the specified *key*.

### 4.1 File menu

These commands are used to open the samples to be paired and other file operations. The current menus and the menu commands (non-working commands have a '\*' prefix) are listed below. You can use either the "Edit options" button to popup the Options Window editor to change the input samples or the (**File menu | Open Rsample**) and (**File menu | Open Sample**) commands.

- **Set project directory** - set the new default project directory and create the new directory and (batch/, cache/, ppx/, rdbms/, tmp/ and xml/) subdirectories if needed. See the -projDir:new\_project-directory switch that you can use to do this on startup.  
-----
- **Open landmark file** - open a landmark database. Ask to save current landmark database if you have made any changes.
- **New landmark file** - create a new landmark database. Ask to save current landmark database if you have made any changes.
- **Rename landmark file** - rename the current landmark database. Ask to save current landmark database if you have made any changes.  
-----
- **Open Rsample** - specify the reference sample from samples in the current accession database.
- **Open Sample** - specify the current sample from samples in the current accession database.  
-----
- **Remove Sample-pair from Landmark DB** - remove the current (Rsample,Sample) pair from the landmark database.  
-----
- **Save Landmark DB** - explicitly save the Landmark DB if changes were made. Otherwise, it is saved when you press "Done".  
-----
- **Clear report** - clear the report text area so that you can start a new report that will be easier to cut and paste or do save to a file
- **Save report to a file** - save the report to a text file  
-----
- **UpdateSubmenu** - update Landmark programs and data from open2dprot.sourceforge.net/Landmark server
- **Landmark all files** - to get the latest files

- **Landmark program** - to get the latest program release (Landmark.jar) and other version dependent files from Landmark server
  - **Landmark demo files** - get latest demo project data files
  - **Landmark documentation** - to get the latest documentation (for stand-alone operation)
- 
- **Exit** - exit the program when in GUI mode.

## 4.2 Edit menu

These commands are used to change various defaults. These are saved when you save the state and when you exit the program.

- **Select Rsample from (Rsample,Sample) list in the LMS DB** - select the Rsample from a list of available landmark sets in the current landmark database. This set of Rsample landmarks can be used when doing "template" editing (see below).
  - **Checkbox off Don't edit Rsample landmarks** - prevent the user from changing the set of coordinates for the Rsample landmarks. Normally, you would not edit the Rsample landmarks after their initial definition. This lets you use exactly the same landmarks when landmarking other samples in subsequent sessions.
  - **Checkbox off Use Rsample landmarks as fixed template** - when landmarking a new sample, the landmarks from the template Rsample (see above) will be available for you to match in the Sample (right image). This ensures that all samples will be paired with the same landmarks in the Rsample.
- 
- **Landmark** - popup the Landmark editor window for the current (Rsample,Sample) pair of samples.
- 
- **OptionsSubmenu** - change the command line options
  - **Open options file** - read a new options file (this overrides the previous options file which could be Landmark.properties).
  - **Save options file** - write the current options to the current options file (default Landmark.properties)
  - **SaveAs options file** - write the current options to a new file you specify
  - **Edit options** - popup the an Options wizard window to to edit the switch options and thier parameters, threshold parameter sliders, and input image file
  - **\*Reset default options** - reset options to the initial default

## 4.3 View menu

This menu contains commands to inspect Landmark database data.

- **List Accession Samples** - list the names of all samples in the Accession database. These are samples that are available for you to define as paired samples.
  - **List Landmark Samples** - list the names of all paired samples in the Landmark database (in the running program). These are listed as pairs of (Rsample,Sample) pairs.
- 
- **Show Rsample accession DB data** - list accession data for the currently selected Rsample in report window.
  - **Show Sample accession DB data** - list accession data for the currently selected Sample in report window.
  - **Show landmark DB data** - show Landmark pairs for the current (Rsample,Sample) database in in

report window.

- **Show radii of landmarks** - list of Landmark radii for the current (Rsample,Sample) in report window.  
-----
- **Show Accession DB in popup browser** - show Accession database in a popup XML browser.
- **Show Landmark DB in popup browser** - show Landmark database in a popup XML browser.

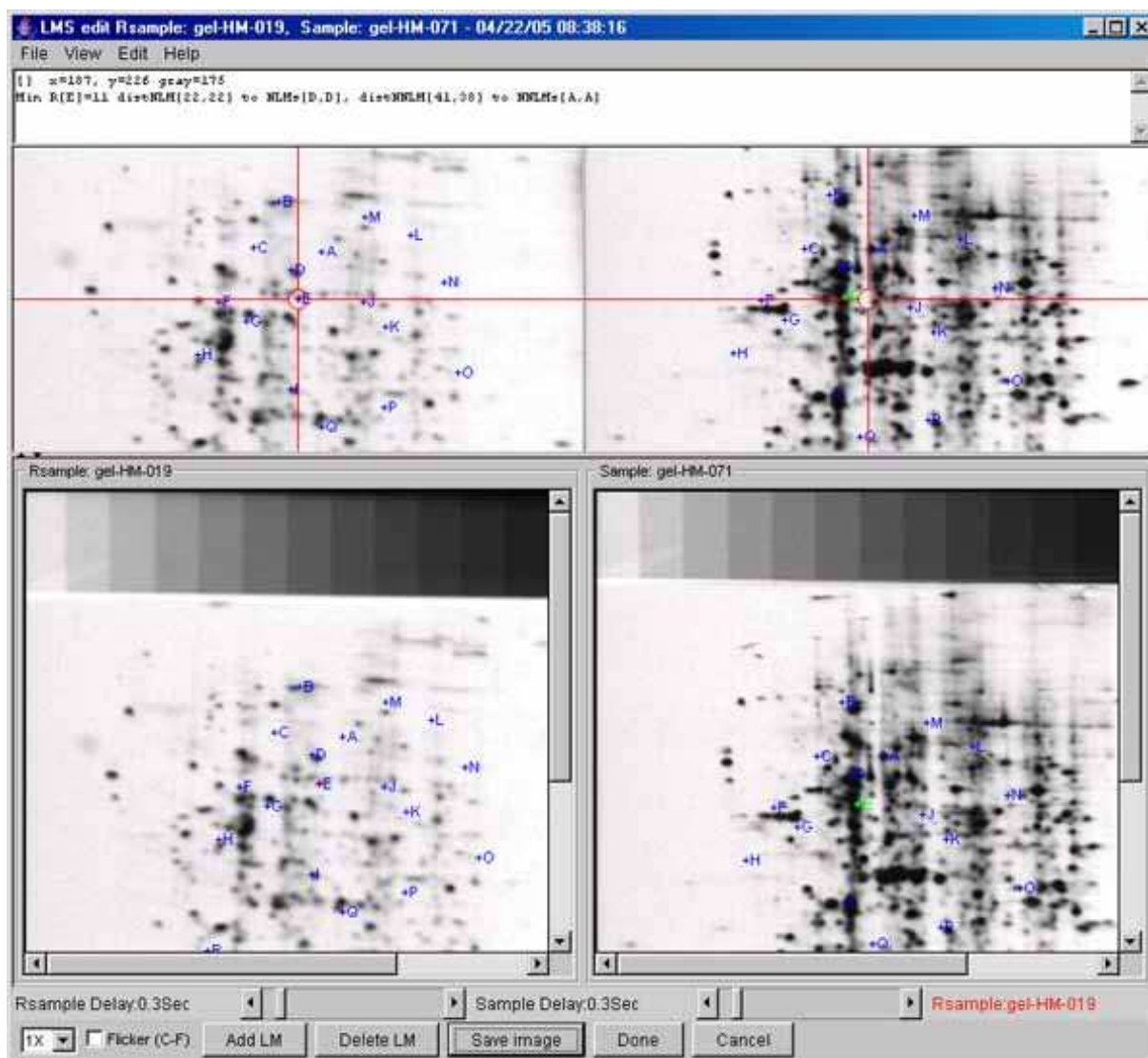
## 4.4 Help menu

These commands are used to invoke popup Web browser documentation on Landmark. Some of the commands will load local documentation in the the GUI report window.

The documentation is kept on the Internet at <http://open2dprot.sourceforge.net/Landmark>. Normally, these help commands should pop up a Web browser that directly points to the Landmark Web page. If your browser is not configured correctly, it may not be able to be launched directly from the Landmark program. Instead, just go to the Web site with your Web browser and look up the information there.

- **Landmark Home** - the [open2dprot.sourceforge.net/Landmark](http://open2dprot.sourceforge.net/Landmark) home page
- **Reference Manual**Submenu - this reference manual
  - **1. Introduction**
  - **2. Running Landmark**
  - **3. Command and Report window**
  - **4. Menus**
  - **5. Popup Editor**
  - **6. Installing Landmark**
  - **7. Command option switches**
  - **8. Demonstrations**
  - **9. References**
- **Sample screen shots** - examples several screen shots
- **Status** - status of the program bugs and future options
- **Revision history** - show latest Landmark release and bugs that were fixed
- **FAQ** - frequently asked questions
- **Contributors** - to the spot pairing program sub-project
- **PDF files** - additional documentation including this manual  
-----
- **Local PDF manual** - popup a PDF browser on the local copy of the PDF document that is also available on the Web site  
-----
- **Open2Dprot home** - home page for the Open2Dprot project
- **License** - the open source license
- **About** - display information about the program

## 5. The popup Landmark Editor Window



**Figure 3. Screen view of the popup Landmark Editor Window.** This lets you create or edit landmarks defined to be common spot positions between the two samples. One of the samples is the Rsample (on the left) and the sample to be landmarked with it on the right (Sample). You select the (Rsample, Sample) in the main Landmark Report Window (see Figure 1). The editor window shows four images. The unmagnified view of Rsample and Sample are on the lower row of images and the magnified corresponding images are on the top row. There is a text area at the top for messages to the user. There is a set of pull-down menus at the top File, View, Edit, and Help. A row of controls on the bottom helps speed up landmarking. There is a 1X to 6X zoom on the left that only zooms the upper images. After you have clicked on corresponding spots in the Rsample and Sample (lower images), select the Flicker checkbox (with (C-F) short-cut) to alternately display the upper Rsample and Sample images in the upper right image. You can use this to verify the spots are probably the same between the two samples. The flicker rate is determined by the delay scrollers at the bottom. The **Add LM** button (with (C-A) short-cut) will add these two selected coordinates as a new landmark spot. Similarly, by first selecting a landmark spot with the mouse and then pressing the **Delete LM** button (with (C-D) short-cut) it will delete this landmark from the list. See the detailed menu commands below for other information on landmarking usage.

The menu bar at the top of the Report Window (see Figure 1) contains four menus. There are also some command buttons at the bottom of the window for convenience that are also menu commands.

1. File menu - to save the landmark set and other related operations.
2. View menu - to change Landmark editor viewing options.
3. Edit menu - to edit landmarks
4. Help menu - popup Web browser documentation on the Landmark Editor window

## 5.1 Editor File menu

- **Save Landmark DB** - explicitly save the Landmark DB if changes were made. Otherwise, it is saved when you press "Done".
- **Checkbox off Log text output to Report window** - copy text output to Report window.  
-----
- **Cancel** - exit the Landmark Editor window without saving any changes you may have made
- **Exit** - exit the Landmark Editor window, but ask to save the landmarks if you made changes.

## 5.2 Editor View menu

- **Checkbox off Flicker Rsample vs Sample (C-F)** - flicker the Rsample and Sample zoom windows (top row) into the upper right zoom window. The flicker rate is determined by the delay scrollers at the bottom.
- **Checkbox off Add landmarks radii overlay** - add half-radii overlays for the defined landmarks
- **Checkbox off Show radii table data for landmark spot** - show additional numeric data for a landmark spot in the text area when you click on a landmark.
- **Checkbox off Use +# instead of <letter> for landmarks** - show landmarks as "+#" rather than "+letter".  
-----
- **Checkbox off Use mouse draw to select spots** - instead of having to click on a spot
- **Checkbox off Add focus box to aid landmarking** - draw a "focus" box around the area you are editing in the Rsample.  
-----
- **Checkbox off Separate Flicker Window** - use a separate flicker window instead of the upper left image windows. You may resize this separate window to see more detail. It is more useful on higher resolution monitors.  
-----
- **Guard region color Submenu** - select the guard region color. This adds a colored region (1/4 of the height or width) to the the image on all four edges so that you can scroll and center the image to data on any of the four edges.
  1. **White**
  2. **Black**
  3. **Gray**
  4. **LightGray**
  5. **DarkGray**
  6. **Cyan**
  7. **Mean image**

## 5.3 Editor Edit menu

- **Add Landmark at current cursor (C-A)** - to add a landmark after you have indicated the coordinates in both the Rsample and Sample.
- **Delete Landmark at current cursor (C-D)** - to delete a landmark after you have indicated the

coordinates in both the Rsample and Sample. If you are not allowed to edit the Rsample then only the Sample landmark will be deleted. See Report Window menu (**Edit | Checkbox on Don't edit Rsample landmarks**).

## 5.4 Editor Help menu

- ...

## 6. Downloading, installing and running Landmark

The installation packages are available for **download** from the SourceForge Files mirror. Look for the most recent release named "Landmark-dist-V.XX.XX.zip". These releases include the program (both as Windows .exe file and a .jar file), required jar libraries, demo data, Windows batch and Unix shell scripts. Download the zip file and put the contents where you want to install the program. Note that there is a Landmark.exe (for Windows program). You might make a short-cut to this to use in more easily starting the program. Alternatively, you can use the sample .bat and .sh scripts to run the program explicitly via the java interpreter. Note that this method assumes that you have Java installed on your computer and that it is at *least* JDK (Java Development Kit) or JRE (Java Runtime Environment) version 1.5.0. If you don't have this, you can download the latest version free from the java.sun.com Website.

There are several ways to run the program. On Windows, you can start Landmark by clicking on the startup icon shown in Figure 6 below. For Unix systems including MacOS-X, you can start Landmark from the command line by running the Landmark.jar file. If your computer is setup to execute jar files, just type the jar file. In both systems, you can specify additional command line arguments in Windows .bat and unix .sh scripts (see demo examples below).

Landmark startup icon

---

**Figure 6. Startup icon for Landmark.** Clicking on the icon starts Landmark. To start Landmark with the graphical user interface, click on the startup icon shown in Figure 6 below - or you can run the demo-Landmark.bat script. For Unix systems including MacOS-X, you can start Landmark from the command line by clicking on the Landmark.jar file or using the demo-Landmark.sh script. These two scripts run the program in batch but use a GUI. You could make short-cuts (Windows) or symbolic-links in Unix to make it easier to start.

### 6.1 Requirements: minimum hardware and software requirements

A Windows PC, MacIntosh with MacOS-X, a Linux computer or a Sun Solaris computer having a display resolution of at *least* 1024x768. We find that a 1024x768 is adequate, but a 1280x1024 screen size much better since you can also see the Popup Report window, Options window, and Landmark, ROI or Calibration window at the same time. At least 30 Mb of memory available for the application is required and more is desirable for comparing large images or performing transforms. If there is not enough memory, it will be unable to load the images, the transforms may crash the program or other problems may occur.

An Internet connection is required to download the program from the Open2Dprot Landmark Web site. New versions of the program and associated demo data will become available on this Web site and can be uploaded to your computer using the various (**File | Update | ...**) menu commands. If you have obtained the installer software that someone else downloaded and gave to you, then you do not need the Internet connection to install the program. We currently distribute Landmark so that it uses up



to 256Mb. See discussion on increasing memory.

## 6.2 Files included in the download

The following files are packaged in the distribution you install. you can periodically a (File | Update from Web server | ... program) menu command to update the files from the open2dprot.sourceforge.net Web server.

- **Landmark.jar** is the Java Archive File for Landmark that is executed when you run Landmark.
- **Landmark.exe** is the Windows executable file that packages Landmark.jar with a java startup. This was packaged using the open-source launch4j (launch4j.sourceforge.net) program.
- **Open2Dprot-Accession.dtd** is the XML .dtd file that can be used with the Landmark database file accession.xml.
- **Open2Dprot-Landmark.dtd** is the XML .dtd file that can be used with the landmark database file landmark.xml.
- **PDF/fullLandmarkDoc.pdf** is a copy of the Landmark Web site for use as a reference manual when using the program off-line. It may not be as up to date as the one on the web site but you can do a (File | Update from Web server | Landmark documentation) menu command to update it.
- **jai\_core.jar** is the core Java runtime from SUN's Java Advanced Imaging (JAI) at java.sun.com
- **jai\_codec.jar** is the JAI tiff file reader from SUN's Java Advanced Imaging JAI at java.sun.com
- **xercesImpl.jar** is the Xerces 2 XML SAX/DOM XML library from Apache xml.apache.org
- **xml-apis.jar** is the Xerces 2 XML SAX/DOM XML library from Apache xml.apache.org
- **license/** is contains the Mozilla.1.1.html and LEGAL.txt files for Landmark and the release-license-jai\_files for the Java Advanced Imaging .jar files used by the TIFF file reader.
- **demo/** is a demonstration project directory containing the the following subdirectories:
  1. **ppx/** directory holds sample image files (4 images)
  2. **tmp/** directory holds generated image files
  3. **xml/** directory that the Landmark DB, landmark DB, input SSF and SPF output filesYou can do a (File | Update from Web server | Landmark demo files) menu command to update it.

## 7. List of the command line switches

The command line usage is:

```
Landmark -rsample:reference-sample -sample:sample [< optional switches >]
```

where the order of arguments is not relevant. In the following list, items in **bold** are specific values which must be used (e.g., for -inputFormat:{**X** | **T**}), whereas *variable* values in *italics* indicate that a numeric value for that variable should be used. Some switches have several alternate fixed choices in which case this indicated as a list of bolded items inside of a set of '{...}' with '|' separating the items. You must pick one of the items and do not include the '{}' brackets. Also, do NOT include any extra spaces in the arguments of the switch - it will be counted as if it were another switch.

### Command line switches

```
-accessionFile:accFile to override the default accession
  database file. (Default is -noaccessionFile:accession.xml).

-backupDatabases makes backups of the Landmark and landmark databases "+
  "if they are edited. (Default is -commutativeLMS).

-commutativeLMS lets you search the landmark database for sample pairs
```

where the Rsample and Sample are swapped. (Default is -commutativeLMS).

-debugBits:bits,optLandmarkNbr dumps various conditional debugging parameters onto the report window. The 'bits' are the debug bits specified as either octal or decimal and enable particular debugging output if the program was compiled with debugging enabled. (Default is -nodebug).

-default sets the default switches to a specific configuration:+

- nodemo
- inputFormat:X -projDir:demo/,
- LandmarkDB:Landmark.xml.

This disables -demo if it was set. (Default -nodefault).

-demo sets the default switches and sample input sample to a specific configuration. This may be overridden by turning off the -demo switch in the Options Wizard.

-dtd adds the XML DTD file (Open2Dprot-SPF.dtd) in the output XML if -spfFormat:X is set. (Default is -nodtd).

-gui to start the spot pairing with a popup Graphical User Interface rather than in batch mode. Landmark does not run in batch mode. This captures messages from Landmark. You can then cut and paste the results or save it to a text file. The GUI is also used to change the switch options, re-run the spot pairing and view images after each analysis. (Default is -gui).

-inputFormat:<accMode> defines the input formats for the Landmark database file accMode). Where mode is: 'F' for full tab-delimited data that includes 2 files: the list of spots, parameters and statistics. The 'T' for tab-delimited (.txt) data, and 'X' for XML (.xml) data. (Default is -noinputFormat:X,X).

-landmarkFile:lmsFile to override the default landmark database file. (Default is -nolandmarkFile:landmark.xml).

-projDir:alternate project directory path to specify the project directory to use instead of the default 'demo/' file in the installation directory. (Default is -noprojDir).

-propertiesFile:alternate 'Landmark.properties' file to specify the alternate startup properties file to use instead of the default 'Landmark.properties' file. (Default is -nopropertiesFile).

-rsample:<Rsample file> is name of the reference sample.

-sample:<Sample file> is the name of the Sample to be paired with the Rsample.

-timer enables a timer to capture processing times for each step. (Default is -notimer).

-update:{all | program | demo | doc} specifies that all of the Landmark files, the program jar files, the documentation files or the demonstration files should be updated from the Open2Dprot Web server. The program should be exited and restarted after updating the program for this to take effect. (Default is -noupdate).

-usage prints all of the switches. (Default is -nousage).

## 7.1 Examples of some typical sets of switches

The following shows a few examples of useful combinations of command line switches.

Any case-independent switch may be negated by preceeding it with a 'no' eg. '-notimer'.

The command line syntax used to invoke it is:

```
Landmark [< opt.-switches >]
```

The following examples using switches might be useful:

```
Landmark -gui -project:demo/  
# Start Landmark and load the Landmark database from the demo project.  
  
Landmark -rsample:gel-HM-019 -sample:gel-HM-071 -gui -project:demo/  
# Start Landmark with two samples already in the Landmark database
```

## 8. Demonstrations

\*\*\* BEING REWRITTEN \*\*\*

### 8.1 Examples - samples of screen shots

To give the flavor of running the spot pairing program, we provide a few screen shots of the graphical user interfaces and some images generated by the program.

You can these images in the list below or view all of the screen shots in a single Web page.

- 1. Initial Report Window
- 2. Initial command-line options tool
- 3. Popup Landmark editor

### 8.2 Example - output of the Report Window for ...

The following Report Window output was generate for the images in the above example.

### 8.3 Examples - using command line processing for batch

It is possible to run the Landmark from the command line in your operating system. We give two examples doing this. The first example shows a script for the Microsoft Windows batch (.bat) file for processing 3 images with the same reference image demo-Landmark.bat file (available on the Files Mirror. The second example shows the same commands in a shell script for a Unix operating system (Linux, MacOS, Solaris, etc.) is available at from the Files mirror at demo-Landmark.sh.

The data for these scripts is in the **demo/** directory available with the installation which also includes the scripts. Alternatively, you can download the demo data from the Files Mirror as Demo.zip.

The installation packages are available from the Files mirror under the Landmark releases.

### 8.3.1 Examples - batch processing under Microsoft Windows

```
REM File: demo-Landmark.bat - interactively define landmark spots for each of 3 samples
REM when paired with the reference sample.
REM This example assumes that all .jar files listed below and demo/ directory are
REM in the current directory. Modify for other situations.
REM
REM The JDK should be installed and version 1.4 or later is required.
REM You can download the latest JDK from http://java.sun.com/
REM
REM The files needed are listed below:
REM JAR files required and mentioned in manifest:
REM     xml-apis.jar xercesImpl.jar jai_codec.jar jai_core.jar O2Plib.jar
REM
REM demo image files:
REM     demo/ppx/gel-HM-019 (Reference sample)
REM     demo/ppx/gel-HM-071
REM     demo/ppx/gel-HM-087
REM     demo/ppx/gel-HM-096
REM Accession database file is in:
REM     demo/xml/accession.xml
REM Landmark database file is in:
REM     demo/xml/landmark.xml
REM Sample Spot-list files (SSF) are in:
REM     demo/xml/gel-HM-019-SSF.xml
REM     demo/xml/gel-HM-071-SSF.xml
REM     demo/xml/gel-HM-087-SSF.xml
REM     demo/xml/gel-HM-096-SSF.xml
REM Generated images are saved in:
REM     demo/tmp/
REM
REM P. Lemkin $Date$

echo "demo-Landmark.bat"

pwd
date /T

java -Xmx256M -jar Landmark.jar -demo -project:demo\ -sample:gel-HM-071 -rsample:gel-HM-019
java -Xmx256M -jar Landmark.jar -demo -project:demo\ -sample:gel-HM-087 -rsample:gel-HM-019
java -Xmx256M -jar Landmark.jar -demo -project:demo\ -sample:gel-HM-096 -rsample:gel-HM-019

echo "-- Finished landmarking the samples ---"
date /T
```

### 8.3.2 Examples - batch processing under Unix (or MacOS-X)

Because java is relatively operating system independent, the same java command lines are used with the "\" changed to "/", "REM" changed to "#", and "DATE/T" to "date" from Windows to Unix script and file path conventions.

```
#!/bin/sh
# File: demo-Landmark.sh - interactively define landmark spots for each of 3
# samples when paired with the reference sample.
# This example assumes that all .jar files listed below and demo/ directory are
# in the current directory. Modify for other situations.
#
# JAR files required and mentioned in manifest:
#     xml-apis.jar xercesImpl.jar jai_codec.jar jai_core.jar O2Plib.jar
#
# The files needed are listed below:
# JAR files required and mentioned in manifest:
#     xml-apis.jar xercesImpl.jar jai_codec.jar jai_core.jar O2Plib.jar
#
# demo image files:
#     demo/ppx/gel-HM-019 (Reference sample)
#     demo/ppx/gel-HM-071
#     demo/ppx/gel-HM-087
#     demo/ppx/gel-HM-096
# Accession database file is in:
#     demo/xml/accession.xml
```

```

# Landmark database file is in:
#     demo/xml/landmark.xml
# Sample Spot-list files (SSF) are in:
#     demo/xml/gel-HM-019-SSF.xml
#     demo/xml/gel-HM-071-SSF.xml
#     demo/xml/gel-HM-087-SSF.xml
#     demo/xml/gel-HM-096-SSF.xml
# Generated images are saved in:
#     demo/tmp/
#
# P. Lemkin $Date$

echo "demo-Landmark.sh"

pwd
date

java -Xmx256M -jar Landmark.jar -demo -project:demo/ -sample:gel-HM-071 -rsample:gel-HM-019
java -Xmx256M -jar Landmark.jar -demo -project:demo/ -sample:gel-HM-087 -rsample:gel-HM-019
java -Xmx256M -jar Landmark.jar -demo -project:demo/ -sample:gel-HM-096 -rsample:gel-HM-019

echo "-- Finished landmarking the samples ---"

date

```

## 9. Landmark References

These papers (a subset of the GELLAB-II papers), reference the GELLAB-II spot pairing program. The Open2Dprot Java-language Landmark program was other Open2Dprot programs and concepts were used from the old GELLAB-II C-language program as well as from code from the MAExplorer and Flicker projects. This program will be replaced with a full XML editor that will make use of the new MIAPE standard. New Java code was added as well. Although Landmark has been enhanced in many ways, the basic algorithm is similar so these papers may be useful for more details on the algorithm.

1. Lipkin, L.E., Lemkin, P.F. (1980) Database techniques for multiple PAGE (2D gel) analysis. *Clinical Chemistry* **26**, 1403-1413.
2. Lemkin, P., Lipkin, L. (1981) GELLAB: A computer system for 2D gel electrophoresis analysis. I. Segmentation and preliminaries. *Computers in Biomedical Research* **14**, 272-297.
3. Lemkin, P., Lipkin, L. (1981) GELLAB: A computer system for 2D gel electrophoresis analysis. II. Pairing Spots. *Computers in Biomedical Research* **14**, 355-380.
4. Lester, E.P., Lemkin, P.F., Lipkin, L.E. (1981) New Dimensions in Protein Analysis - 2D Gels Coming of Age Through Image Processing, Invited paper, *Analytical Chemistry* **53**, 390A-397A.
5. Lemkin, P.F., Lipkin, L.E. (1983) Database Techniques for 2D Electrophoretic Gel Analysis, in Computing in Biological Science, Elsevier/North-Holland, M. Geisow, A. Barrett (eds), 181-226.
6. Lemkin, P.F., Lipkin, L.E., Lester, E.P. (1982) Extensions to the GELLAB 2D Electrophoresis Gel Analysis System. Paper given at "Clinical Applications of 2D Electrophoresis", Mayo Clinic, Nov. 15-18, 1981. *Clinical Chemistry* 840-849.
7. Lemkin, P.F., Lester, E.P. (1989) Database and Search Techniques for 2D Gel Protein Data: A Comparison of Paradigms For Exploratory Data Analysis and Prospects for Biological Modeling, *Electrophoresis* **10**(2): 122-140.

8. Lemkin, P.F. (1989) GELLAB-II, A workstation based 2D electrophoresis gel analysis system, in proceedings of Two-Dimensional Electrophoresis T. Endler, S.Hanash (Eds), Vienna Austria, Nov 8-11, 1988, VCH Press, W.Germany. pp 53-57.
  9. Lemkin, P.F., Rogan, P. (1991) Automatic Detection of noisy spots in two-dimensional Southern Blots, *Applied and Theoretical Electrophoresis* **2**, 141-149.
  10. Myrick, J.E., Lemkin, P.F., Robinson, M.K., Upton, K.M. (1993) Comparison of the BioImage Visage(TM) 2,000 and the GELLAB-II two-dimensional electrophoretic analysis systems. *Applied and Theoretical Electrophoresis* **3**, 335-346.
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[open2dprot.sourceforge.net/Landmark](http://open2dprot.sourceforge.net/Landmark)  
Powered by SourceForge Logo

Revised:  
05/31/2006

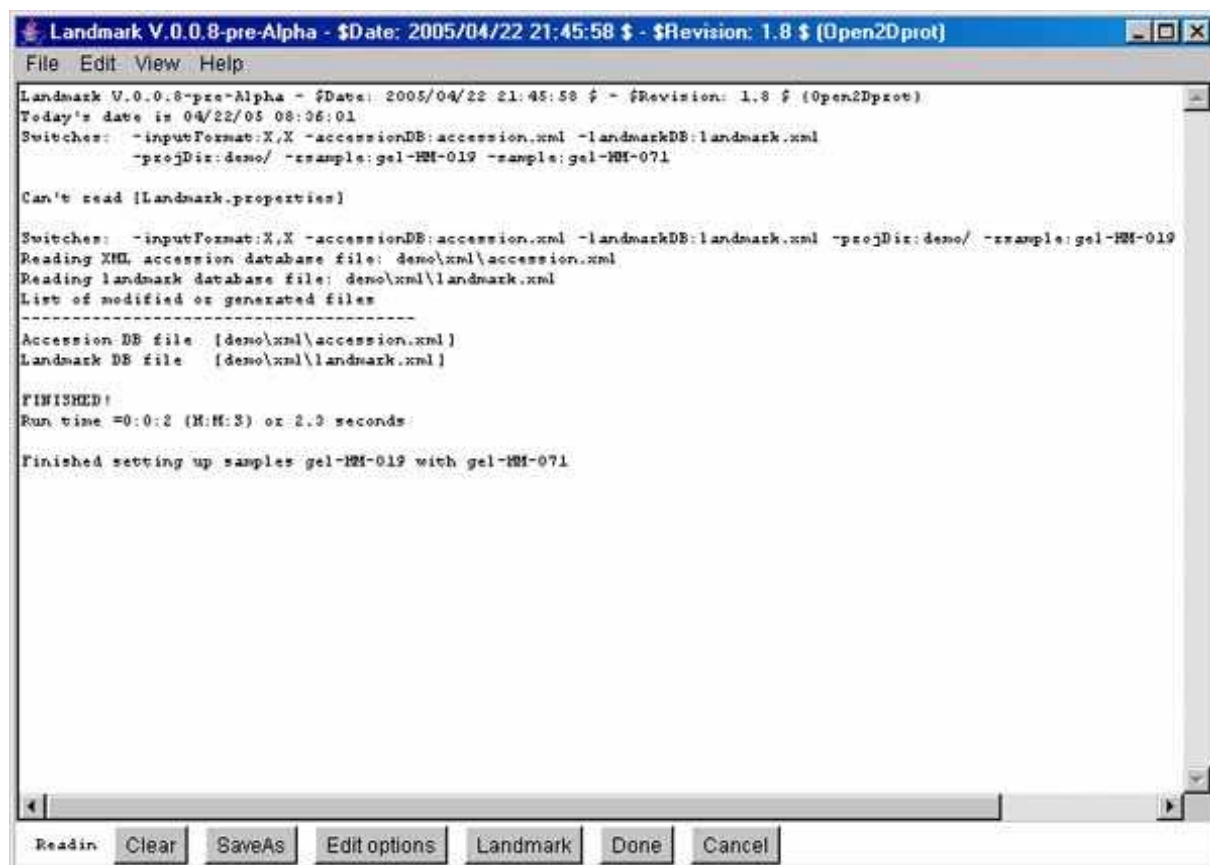
Sample Screen Shots for Landmark program - pre-alpha version

## **Sample Screen Shots for Landmark program - pre-alpha version**

To give the flavor of running the spot pairing program, we provide a few screen shots of the graphical user interface generated by the program.

1. Initial user interface window
2. Initial command line options tool
3. Using Landmark data popup editor

### **1. Initial Report Window**



---

## 2. Initial command line options tool

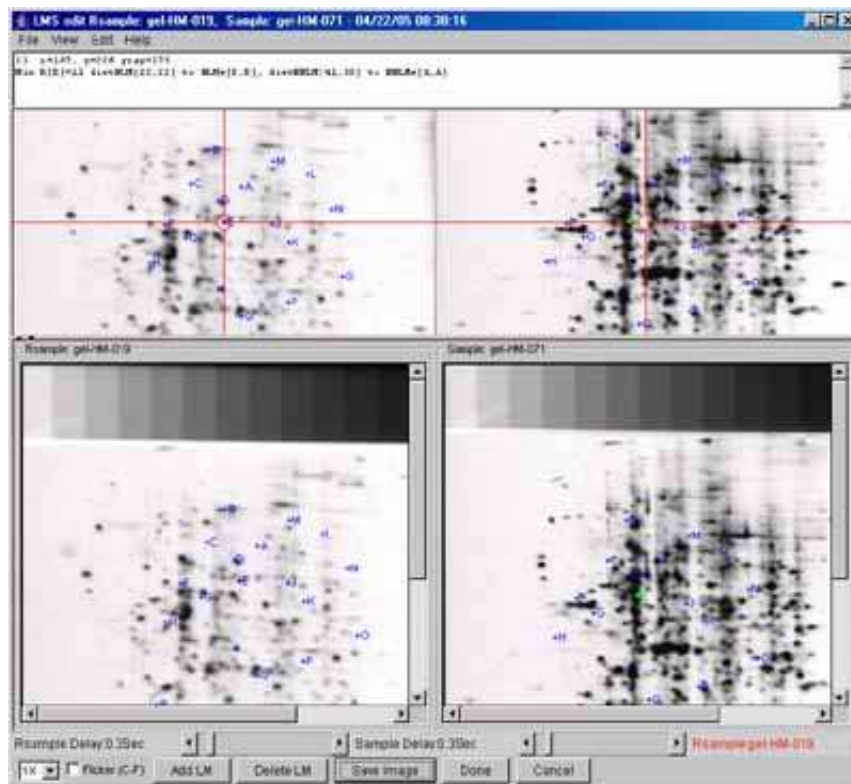
**Enter sample image and select switch options**

Change the startup parameters, then press 'Set new options' button to save them.  
 At that point, you can press 'Pair spots' to pair the Rsample and Sample with the new parameters. You may also enter new Rsample and Sample data file names and/or edit switch options and threshold sliders.

<input checked="" type="checkbox"/> -accessionFile	accession.xml
<input checked="" type="checkbox"/> -backupDatabases	---
<input type="checkbox"/> -commutativeLMS	---
<input type="checkbox"/> -debugBits	0.0
<input type="checkbox"/> -default	---
<input checked="" type="checkbox"/> -demo	---
<input type="checkbox"/> -dtd	---
<input checked="" type="checkbox"/> -inputFormat	X,X
<input checked="" type="checkbox"/> -landmarkFile	landmark.xml
<input checked="" type="checkbox"/> -projDir Project directory <input type="button" value="Browse dir"/>	demo\
<input type="checkbox"/> -propertiesFile Properties file <input type="button" value="Browse file"/>	Landmark.properties
<input checked="" type="checkbox"/> -rsample Rsample file <input type="button" value="Browse file"/>	gel-HM-018
<input checked="" type="checkbox"/> -sample Sample file <input type="button" value="Browse file"/>	gel-HM-071
<input type="checkbox"/> -separateFlickerWindow	---
<input type="checkbox"/> -timer	---
<input type="checkbox"/> -update <input type="button" value="program"/>	program
<input type="checkbox"/> -usage	---

### 3. Using popup Landmark data editor





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[open2dprot.sourceforge.net/Landmark](http://open2dprot.sourceforge.net/Landmark)

Revised:  
10/04/2005

Seg2Dgel Jar Version

## Landmark Jar Version

There are two versions of the Landmark program available on Open2Dprot at the server

<http://open2dprot.sourceforge.net/Landmark>

The first is the installer version that you download from the Web server. The second is the possibly latest version that you update by having Landmark copy the Landmark.jar file from the Web server using the (File | Update | Landmark program) menu command. After you do the update, you need to restart Landmark to use it.

Until further notice, Landmark is to be considered pre-alpha-level code until it is officially released. This means that there may be some functionality not fully implemented, that works incorrectly, or that has changed. New commands and functionality are in the process of being added. Please report problems and suggestions to us.

Landmark version 0.17

Landmark - (PDF) documents

# Landmark - Acrobat logo (PDF) documents

There are a number of Adobe Acrobat PDF formatted documents and slide shows available for the Landmark project.

If you do not have Adobe Acrobat, you can download it for free from Adobe. Get Acrobat reader The following lists some of the PDF documents you may download. As others are created, they will be added to this list.

1. Landmark Overview slide show for use as a printable document.
2. Landmark Overview slide show for use as a printable document (2 panels/page).
3. Landmark Overview slide show for use as a printable document (6 panels/page).
4. This Landmark Web site as a PDF document for use as a printable document

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Landmark is a contributed program available at  
[open2dprot.sourceforge.net/Landmark](http://open2dprot.sourceforge.net/Landmark)

Revised:  
10/04/2005  
004

Landmark Revision History

## Landmark Revision History

This describes the revision history of the Landmark program of released or soon to be released versions. Generally, only the most recent versions are kept on the Web site (see Version). Documentation for unresolved bugs and requested functionality is found in the status Web page.

- **V.0.17 05-31-2006:** Get (File | Updates) from main server jar/, demo/, dtd/, scripts/ etc. directories.
- **V.0.16 12-21-2005:** fixed bug which limited number of landmarks. This was a O2Plib.db.DbLM and O2Plib.db.LMset change. Added menu option to display landmarks as "+#" rather than "+letter". It defaults to "+#" if there are more than 52 landmarks. Change color of displayed landmarks to magenta from blue which should be easier to see.
- **V.0.15 11-07-2005:** Changed installer paradigm to use launch4j. Also, new manifest for Landmark.jar file that now includes the Class-Path so the demo .bat and .sh scripts were simplified in this release. The (**Edit menu | Resize memory limits**) command was deprecated - see Increasing or decreasing the allowable memory used by Landmark in the Reference manual.
- **V.0.12 06-24-2005:** Added image guard region for comparing spots on the edges of the images. This allows you to center edge spots in the zoom and flicker windows.
- **V.0.11 06-22-2005:** Fixed bug introduced with zoom that swapped Rsample and Sample coordinates. Corrected now.

- **V.0.10 06-18-2005:** Enabled Guard region processing and added (Edit | [IMAGE] Add guard region to edges of images) checkbox menu command. This adds a white region (1/4 of the height or width) to the the image on all four edges so that you can scroll and center the image to data on any of the four edges.
- **V.0.0.8 04-22-2005:** Initial release Web site version.
- **V.0.0.6 02-17-2005:** Pre-release version. This version is buggy. The calibration options do not work correctly. However, it does allow adding a sample and defining image ROIs.

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Revised:  
05-31-2006

Landmark Program Status

## Landmark Program Status

The following are known bugs, suggested features, functions that are being debugged or being developed. Documentation for resolved bugs is found in the revision history Web page.

- Revision history
- Known bugs
- Suggested Features/FAQ

## Known Bugs Known bugs

The neumonic *GGGR* is shorthand for "Grep, Get Gestault and Refactor".

- **V.0.0.6 02-17-2005:** Initial version.

## Suggested Features Suggested features

- **V.0.0.6 02-17-2005:** [TODO]

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Revised:  
10/04/2005